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4 April 2002



PATENT APPLICATION
Docket No.: 0054.1087-005

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Barbara A. Gilchrest, Mina Yaar and Mark Eller

Application No.: 09/018,194 Group: 1647

Filed: February 4, 1998 Examiner: S. Wegert

For: Methods of Inducing Hair Growth and Coloration

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CERTIFICATE OF MAILING	
I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to Assistant Commissioner for Patents, P.O. Box 2327, Arlington, VA 22202	
on <u>4/8/02</u>	<u>K. Bastarach</u> Signature
_____ <u>Kathleen Bastarach</u> Typed or printed name of person signing certificate	

DECLARATION OF BARBARA A. GILCHREST, M.D., UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
P. O. Box 2327
Arlington, VA 22202

Sir:

I, Barbara A. Gilchrest, M.D. of 343 Commercial Street, Union Wharf # 27, Boston, Massachusetts 02109, hereby declare that:

1. I am a co-inventor of the subject matter described and claimed in Patent Application No. 09/018,194 entitled "Methods of Inducing Hair Growth and Coloration" filed on February 4, 1998.

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2. Keratinocytes grown in cell culture provide a reasonable model to allow for tests of various treatments in hair growth, because the cells lining the hair follicles and giving rise to the hair shaft are keratinocytes. Cell types found in skin have long been grown in tissue culture and have been used as experimental models for the growth and responsive behaviors of cells of the hair follicle. As examples of the other researchers' use of cell cultures as models of hair follicles, two journal articles are enclosed. The first, Detmar *et al.* (Detmar, M. *et al.*, *J. Invest. Dermatol. 101 (1 Suppl.):130S-134S, 1993; Exhibit A*), describes the culturing of keratinocytes and their use in studies on regulation of the hair growth cycle. The second, Moll (Moll, I., *J. Invest. Dermatol. 105(1): 14-21, 1995; Exhibit B*), describes a study of keratinocytes in which it was found that the keratinocytes grown in culture were capable of differentiation, as required for hair formation.
3. In experiments described in the patent application, it was found that nerve growth factor promotes the survival of keratinocytes in culture. See Example 10, page 41, lines 7-30. In other experiments described in the patent application, it was found that peptides containing the KGA amino acid sequence, including the peptide having amino acid sequence CATDIKGAEC (SEQ ID NO:9), promoted the survival of p75^{NTR}-NIH 3T3 fibroblast cells in culture. See Example 18 on page 48, line 7 to page 49, line 2, and Figures 12A-12C. In more recent experiments described below using biopsies of mouse skin containing whole hair follicles, the same peptide had the effect predicted by the experiments in cell culture promoting cell growth and survival, of promoting the retention of hair and prolongation of the anagen (growth phase) portion of the hair cycle by a delay in catagen (regression phase).
4. Biopsies of mouse skin during the early stages of catagen were maintained in organ culture at an air-liquid interphase on gelatin gels (Li, *et al. In Vitro Cell Dev. Biol.* 28:695-698, 1992), and provided with 100 μ M p75^{NTR} antagonistic cyclic peptide SEQ ID NO:9 (CATDIKGAEC) or diluent as control. At the end of the incubation period (48

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hours), skin fragments were washed repeatedly in PBS at 4°C, fixed in 4% paraformaldehyde, and embedded in paraffin for routine histology and histomorphometry. The percent of hair follicles in each defined catagen stage was calculated in 8-10 biopsies per group at 400x magnification using a Zeiss Axioscope microscope, as described previously (Paus *et al.*, *J. Invest. Dermatol.* 103:143-147, 1994). The cyclic peptide delayed catagen development of hair, showing that blocking p75^{NTR} activation is associated with delay of catagen initiation and establishing that the cyclic peptide provides a means for affecting hair growth (*p<0.05). See Figure 1: p75^{NTR} antagonistic cyclic peptide retards catagen development.

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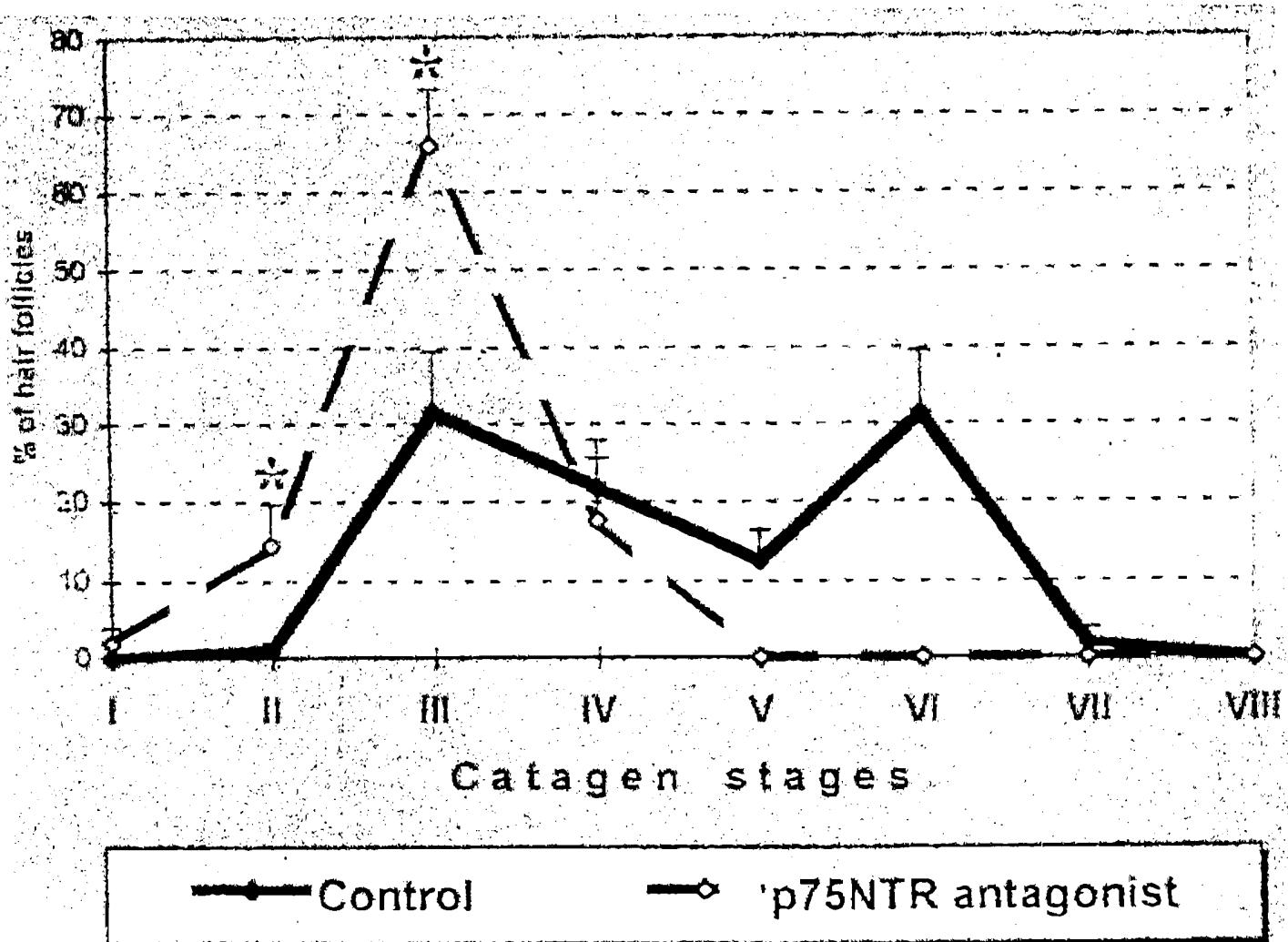


Figure 1

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5. Biopsies of mouse skin during the early stages of catagen were maintained in organ culture as above. Cultures were supplemented with either brain derived neurotrophic factor (BDNF), a neurotrophin that binds p75^{NTR} to induce catagen progression; p75^{NTR} antagonistic cyclic peptide CATDIKGAEC; both BDNF and p75^{NTR} antagonistic cyclic peptide; or diluent alone as control. As expected, compared to diluent treated control skin specimens, the cyclic peptide delayed catagen progression and the majority of cyclic peptide treated hair follicles were in early catagen stage (II). Also as expected, compared to diluent treated control, organ cultures maintained in the presence of BDNF progressed faster into catagen and the majority of hair follicles were in advanced catagen stages (III and IV). However, the cyclic peptide abrogated the BDNF effect, resulting in the majority of the hairs present being in the early catagen stages, similar to those organ cultures treated with the cyclic peptide alone. This experiment confirms that the cyclic peptide can be used to inhibit neurotrophin induced hair cycle regression (*p<0.05). See Figure 2: p75^{NTR} antagonistic cyclic peptide blocks BDNF initiated catagen development.

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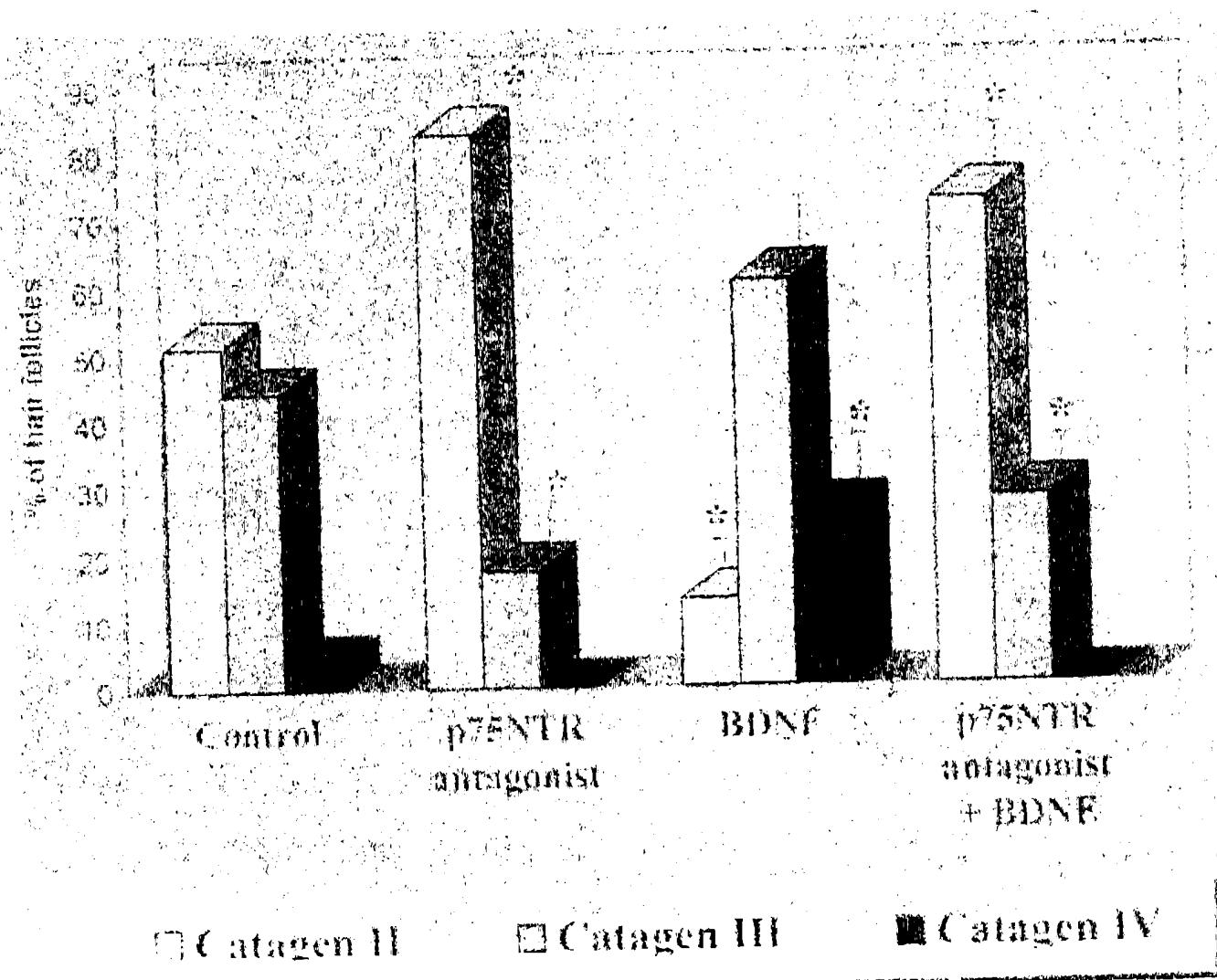


Figure 2

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6. The results of the experiments using cells in culture parallel the results of the experiments using biopsies of mouse skin maintained in organ culture. Therefore, the experiments in which cyclic peptide SEQ ID NO:9 and other ligands of p75^{NTR} were found to block apoptosis in keratinocytes and fibroblasts are predictive of the results that are to be found in hair follicles.
7. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.



Barbara A. Gilchrest, M.D. 4/5/02

Barbara A. Gilchrest, M.D.

Date

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EXHIBIT

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